2/2/



OIPE

## ENTERED

RAW SEQUENCE LISTING

PATENT APPLICATION: US/09/876,796B

DATE: 02/28/2002 TIME: 12:22:24

Input Set : A:\Rb125seq.txt

Output Set: N:\CRF3\02282002\I876796B.raw

```
3 <110> APPLICANT: Horwath, K. L. , Easton, C. M. , and Myers, K. L.
      5 <120> TITLE OF INVENTION: Nucleic Acid Sequences Encoding Type III Tenebrio
              Antifreeze Proteins and Method for Assaying Activity.
      8 <130> FILE REFERENCE: RB-125-PCT
C--> 10 <140> CURRENT APPLICATION NUMBER: US/09/876,796B
C--> 11 <141> CURRENT FILING DATE: 2001-08-08
     13 <150> PRIOR APPLICATION NUMBER: US 60/210,446
     14 <151> PRIOR FILING DATE: 2000-06-08
     16 <160> NUMBER OF SEQ ID NOS: 48
     18 <170> SOFTWARE: Microsoft Word
     20 <210> SEQ ID NO: 1
     21 <211> LENGTH: 19
     22 <212> TYPE: PRT
     23 <213> ORGANISM: Tenebrio molitor
     25 <223> OTHER INFORMATION: N-terminal sequence of protein Tm 12.86
     27 <400> SEQUENCE: 1
     28 Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser Lys Glu Cys
     29 1
                                            10
     31 Gln Gln Val
                19
     34 <210> SEQ ID NO: 2
     35 <211> LENGTH: 576
     36 <212> TYPE: DNA
     37 <213> ORGANISM: Tenebrio molitor
     39 <223> OTHER INFORMATION: Non-his-tagged, signal plus, Tm 13.17
     41 <400> SEQUENCE: 2
     42 gtggatccaa agaattcggc acqaqactac taag atg aag ttg ctc
                                                                          46
     43
                                              Met Lys Leu Leu
     44
                                                           -15
     46 tgt tgt cta atc tcc ctc att ctg ttg gtc aca gtt cag gcc ctg
                                                                          91
     47 Cys Cys Leu Ile Ser Leu Ile Leu Leu Val Thr Val Gln Ala Leu
                        -10
     50 acc gag gca caa att gag aaa ctg aac aag atc agc aaa aaa tgt
                                                                         136
     51 Thr Glu Ala Gln Ile Glu Lys Leu Asn Lys Ile Ser Lys Lys Cys
                                        10
     54 caa aat gaa agt gga gtg tcg caa gag atc ata acc aaa gct cgc
                                                                         181
     55 Gln Asn Glu Ser Gly Val Ser Gln Glu Ile Ile Thr Lys Ala Arg
     56
                    20
                                        25
     58 aac ggt gac tgg gag gac gat cet aaa etg aaa ege caa gtt ttt
                                                                         226
     59 Asn Gly Asp Trp Glu Asp Asp Pro Lys Leu Lys Arg Gln Val Phe
                                        40
     62 tgc gtg gcc agg aac gcc ggt ctg gcc acg gaa tcg gga gag gtg
                                                                         271
```

63 Cys Val Ala Arg Asn Ala Gly Leu Ala Thr Glu Ser Gly Glu Val

RAW SEQUENCE LISTING DATE: 02/28/2002 PATENT APPLICATION: US/09/876,796B TIME: 12:22:24

Input Set : A:\Rb125seq.txt
Output Set: N:\CRF3\02282002\1876796B.raw

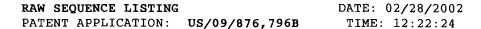
64 50 55 60												
66 gtg gtc gac gtg ttg agg gag aag gtg,agg aag gtc act gac aac	316											
67 Val Val Asp Val Leu Arg Glu Lys Val Arg Lys Val Thr Asp Asn 68 65 70 75												
70 gac gaa gaa act gag aaa atc atc aat aag tgc gcc gtc aag aga	361											
71 Asp Glu Glu Thr Glu Lys Ile Ile Asn Lys Cys Ala Val Lys Arg												
72 80 85 90												
74 gat act gtt gaa gag acg gtg ttc aat act ttc aaa tgt gtc atg	406											
75 Asp Thr Val Glu Glu Thr Val Phe Asn Thr Phe Lys Cys Val Met												
76 95 100 105	449											
8 aaa aac aag cca aag ttc tca cca gtt gat tga accaccacga 4 9 Lys Asn Lys Pro Lys Phe Ser Pro Val Asp												
80 110 115												
82 ctagtagatg gttcaaatgg tgtgctttac atataaaaat aaagtgtttc 499												
84 tgatgtaaaa aaaaaaaaa aaaaaaaaaa aactcgagag tattctagag 549												
86 cggccgcggg cccatcgttt tccaccc 576												
89 <210> SEQ ID NO: 3												
90 <211> LENGTH: 134												
91 <212> TYPE: PRT												
92 <213> ORGANISM: Tenebrio molitor												
94 <223> OTHER INFORMATION: Precursor Protein for Tm 13.17 96 <400> SEOUENCE: 3												
97 Met Lys Leu Cys Cys Leu Ile Ser Leu Ile Leu Leu Val Thr Val												
98 -15 -10 -5												
100 Gln Ala Leu Thr Glu Ala Gln Ile Glu Lys Leu Asn Lys Ile Ser Lys	5											
101 1 5 10												
103 Lys Cys Gln Asn Glu Ser Gly Val Ser Gln Glu Ile Ile Thr Lys Ala	ì											
104 15 20 25 30												
106 Arg Asn Gly Asp Trp Glu Asp Asp Pro Lys Leu Lys Arg Gln Val Phe	<b>3</b>											
107 35 40 45 109 Cys Val Ala Arg Asn Ala Gly Leu Ala Thr Glu Ser Gly Glu Val Val	•											
110 50 55 60	-											
112 Val Asp Val Leu Arg Glu Lys Val Arg Lys Val Thr Asp Asn Asp Glu	ı											
113 65 70 75												
115 Glu Thr Glu Lys Ile Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val	-											
116 80 85 90												
118 Glu Glu Thr Val Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro												
119 95 100 105 110	)											
121 Lys Phe Ser Pro Val Asp 122 115												
122 115 125 <210> SEQ ID NO: 4												
126 <211> LENGTH: 116												
127 <212> TYPE: PRT												
128 <213> ORGANISM: Tenebrio molitor												
130 <223> OTHER INFORMATION: Mature Protein for Tm 13.17												
132 <400> SEQUENCE: 4												
133 Leu Thr Glu Ala Gln Ile Glu Lys Leu Asn Lys Ile Ser Lys Lys Cys	}											
134 1 5 10 15 136 Gln Asn Glu Ser Gly Val Ser Gln Glu Ile Ile Thr Lys Ala Arg Asn												

RAW SEQUENCE LISTING DATE: 02/28/2002 PATENT APPLICATION: US/09/876,796B TIME: 12:22:24

Input Set : A:\Rb125seq.txt

Output Set: N:\CRF3\02282002\1876796B.raw

137				20					25					30		
	Glv	Δen	Trn		Aen	λen	Dro	T.v.c		Lvc	λκα	Gln	Va l		Cys	Val
140	GLY	дър	35	Giu	тэр	кэр	FIO	цуS 40	Deu	пур	AIG	GIII	45	Pne	Cys	Val
	Δla	Δrσ		Δla	Glv	T.211	Δla		Glu	Sor	G1 sz	Glu		17 a 1	Val	Λcn
143	11.1u	50	non	mu	Gry	шец	55	1111	Giu	DCI	СТУ	60	Val	Val	Val	кър
	Val		λνα	Glu	Twe	Val		Lvc	Val	Thr	λαn		λan	C1.,	Glu	mh n
145		пец	Ary	GIU	nys	70	AIG	гуу	Val	1111	75	ASII	ASP	GIU	GIU	
		Tvc	Tlo	т10	λαη		Cvc	71 -	val	T ***	. –	7 an	mb ~	17.0 ]	Glu	80
149	Giu	пуз	116	116	85 85	цуъ	Cys	нта	vai	ьуs	Arg	ASP	TIII	Val	95	GIU
	Thr	W = 1	Dho	Nan		Dho	T 170	Crra	1727		T	7 00	T	D	Lys	Dh.a
152	1111	val	PHE	100	1111	Pne	ьуѕ	Cys	105	Met	ьуѕ	ASII	гĀR		ьуѕ	Phe
	Sar	Pro	Wa 1						103					110		
155	ser	PIO		ASP												
	8 <210> SEQ ID NO: 5															
	9 <211> LENGTH: 481															
	60 <212> TYPE: DNA 61 <213> ORGANISM: Tenebrio molitor															
										,			_		_	
						LION	: NO	n-His	s-tag	gged,	, S19	jna i	plus	s, C.	lone	2.2
				NCE:												
	ggc	acga	gca a		_			ctc 1	_	_		-	-		_	46
167				ľ	Met I	Lys 1		Leu 1	Leu (	Cys I	Phe A			Ala <i>I</i>	Ala	
168								-15					-10			
								ctc								91
	Ile	Val		Gly	Ala	Gln	Ala	Leu	Thr	Asp	Glu		Ile	Gln	Lys	
172			<b>-</b> 5					1				5				
								tgc								136
	Arg		Lys	Ile	Ser	Lys	Glu	Cys	Gln	Gln	Val	Ser	Gly	Val	$\operatorname{Ser}$	
176		10					15					20				
								cgc								181
	Gln		Thr	Ile	Asp	Lys	Val	Arg	Thr	Gly	Val	Leu	Val	Asp	Asp	
180		25					30					35				
								ctc								226
	Pro		Met	Lys	Lys	His	Val	Leu	Cys	Phe	Ser	Lys	Lys	Thr	Gly	
184		40					45					50				
								acc								271
187	Val	Ala	Thr	Glu	Ala	Gly		Thr	Asn	Val	Glu	Val	Leu	Lys	Ala	
188		55					60					65				
								gac								316
	Lys		Lys	His	Val	Ala		Asp	Glu	Glu	Val	Asp	Lys	Ile	Val	
192		70					75					80				
								gcc								361
195	Gln	Lys	Cys	Val	Val	Lys	Lys	Ala	Thr	Pro	Glu	Glu	Thr	Ala	Tyr	
196		85					90					95				
198	gac	acc	ttc	aag	tgt	att	tac	gac	agt	aaa	cct	gat	ttc	tct	cct	406
								Asp								
200		100					105					110				
202	2 att gat taa ttgttttgta tttgactgaa ttttgacaat aaaggtaata												455			
	Ile			-	_		-	-		-						
204		115														



Input Set : A:\Rb125seq.txt

Output Set: N:\CRF3\02282002\1876796B.raw

```
206 tcqttatqta aaaaaaaaa aaaaaa
                                                                   481
209 <210> SEO ID NO: 6
210 <211> LENGTH: 482
211 <212> TYPE: DNA
212 <213> ORGANISM: Tenebrio molitor
214 <223> OTHER INFORMATION: Non-His-tagged, Signal plus, Clone 2.3
216 <400> SEQUENCE: 6
217 ggcacgagca aaa atg aaa ctc ctc ttg tgc ttt qct ttc qcc qcc
                                                                    46
                   Met Lys Leu Leu Cys Phe Ala Phe Ala Ala
219
                               -15
221 atc gtc atc gga gct cag gct ctc acc gac gaa cag ata cag aaa
                                                                    91
222 Ile Val Ile Gly Ala Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys
223
225 agg aac aag atc agc aaa gaa tgc cag cag gtg tcc gga gtg tcc
                                                                   136
226 Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val Ser
                            15
229 caa gag acg atc gac aaa gtc cgc aca ggt gtc ttg gtc gac gat
                                                                   181
230 Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp Asp
233 ccc aaa atg aag aag cac gtc ctc tgc ttc tcg aag aaa act gga
                                                                   226
234 Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr Gly
                            45
237 gtg gca acc gaa gcc gga gac acc aat gtg gag gta ctc aaa gcc
                                                                   271
238 Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys Ala
239
        55
                            60
241 aag ctg aag cat gtg gcc agc gac gaa gaa gtg gac aag atc gtg
                                                                   316
242 Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile Val
                            75
245 cag aag tgc gtg gtc aag aag gcc aca cca gag gaa acg gct tat
                                                                   361
246 Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr
                            90
249 gac acc ttc aag tgt att tac gac agt aaa cct gat ttc tct cct
                                                                   406
250 Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro
                            105
252 att gat taa ttgttttgta tttgactgaa ttttgacaat aaaggtacta
                                                                   455
253 Ile Asp
254
       115
256 tcgttatgaa aaaaaaaaa aaaaaaa
                                                                   482
259 <210> SEO ID NO: 7
260 <211> LENGTH: 133
261 <212> TYPE: PRT
262 <213> ORGANISM: Tenebrio molitor
264 <223> OTHER INFORMATION: Precursor Protein for Tm 12.84, Clones 2.2, 2.3, and 7.5
266 <400> SEQUENCE: 7
267 Met Lys Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala
                -15
                                    -10
270 Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser Lys
271
273 Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp Lys Val
```

RAW SEQUENCE LISTING DATE: 02/28/2002 PATENT APPLICATION: US/09/876,796B TIME: 12:22:24

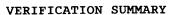
Input Set : A:\Rb125seq.txt

Output Set: N:\CRF3\02282002\1876796B.raw

```
274 15
                         20
                                             25
                                                                  30
276 Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys His Val Leu
279 Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala Gly Asp Thr Asn
282 Val Glu Val Leu Lys Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu
285 Val Asp Lys Ile Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu
                             85
288 Glu Thr Ala Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp
                         100
                                             105
291 Phe Ser Pro Ile Asp
292
295 <210> SEQ ID NO: 8
296 <211> LENGTH: 115
297 <212> TYPE: PRT
298 <213> ORGANISM: Tenebrio molitor
300 <223> OTHER INFORMATION: Mature Protein for Tm 12.84, Clones 2.2, 2.3, and 7.5
302 <400> SEQUENCE: 8
303 Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser Lys Glu Cys
306 Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp Lys Val Arg Thr
307
                                     25
309 Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys His Val Leu Cys Phe
312 Ser Lys Lys Thr Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu
315 Val Leu Lys Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp
316 65
318 Lys Ile Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr
319
                    85
321 Ala Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser
322
                100
                                     105
324 Pro Ile Asp
325
            115
328 <210> SEQ ID NO: 9
329 <211> LENGTH: 481
330 <212> TYPE: DNA
331 <213> ORGANISM: Tenebrio molitor
333 <223> OTHER INFORMATION: Non-His-tagged, Signal plus, Clone 3.4
335 <400> SEQUENCE: 9
336 ggcacgagca aaa atg aaa ctc ctc ttg tgc ttt gct ttc gcc gcc
                                                                    46
337
                   Met Lys Leu Leu Cys Phe Ala Phe Ala Ala
338
                               -15
340 atc gtc atc gga gct cag gct ctc acc gac gaa cag ata cag aaa
                                                                    91
341 Ile Val Ile Gly Ala Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys
344 agg aac aag atc agc aaa gaa tgc cag cag gtg tcc gga gtg tcc
                                                                   136
345 Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val Ser
```



Use of n and / or Xaa has been detected in the Sequence Listing. Review the Sequence Listing to ensure a corresponding explanation is present in the <220> to <223> fields of each sequence using n or Xaa.



DATE: 02/28/2002 TIME: 12:22:25

PATENT APPLICATION: US/09/876,796B

Input Set : A:\Rb125seq.txt

Output Set: N:\CRF3\02282002\I876796B.raw

```
L:10 M:270 C: Current Application Number differs, Replaced Current Application Number
L:11 M:271 C: Current Filing Date differs, Replaced Current Filing Date
L:1880 \ M:341 \ W: \ (46) \ "n" \ or "Xaa" \ used, for SEQ ID#:44
L:1888 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:44
L:1889 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:44
L:1912 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:44
L:1913 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:44
L:1916 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:44
L:1920~M:341~W:~(46) "n" or "Xaa" used, for SEQ ID#:44
L:1934 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45
L:1935 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45
L:1938 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45
L:1939 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45
L:1942 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45
L\!:\!1946 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45
L:1950 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45
L:1954 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45
L:1958 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45
L:1962 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45
L:1966 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45
L:1970 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45
L:1974 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:45
L:1989 \text{ M}:341 \text{ W}: \text{ (46) "n" or "Xaa" used, for SEQ ID#:46}
L:1993 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46
L:1997 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46
L:2001 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46
L:2005 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46
L:2009 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46
L:2013 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46
L:2017 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46
L:2021 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46
L:2025 \ M:341 \ W: (46) "n" or "Xaa" used, for SEQ ID#:46
L:2029 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:46
L:2044 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47
L:2048 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47
L:2052 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47
L:2056 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47
L:2060 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47
L:2064 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47
L:2068\ M:341\ W: (46) "n" or "Xaa" used, for SEQ ID#:47
L:2072 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47
L:2076 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47
L:2080 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47
L:2084 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:47
L:2099 \ M:341 \ W: (46) "n" or "Xaa" used, for SEQ ID#:48
L:2102 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48
L:2105 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48
L:2108 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48
```



## VERIFICATION SUMMARY

DATE: 02/28/2002 PATENT APPLICATION: US/09/876,796B TIME: 12:22:25

Input Set : A:\Rb125seq.txt

Output Set: N:\CRF3\02282002\I876796B.raw

L:2111 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48 L:2114 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48 L:2117 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48 L:2120 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:48